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Natural transformation assay

MB Melanie Blokesch

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An abbreviated version of this protocol was published in eLIFE in Sep 2019

Neighbor predation linked to natural competence fosters the transfer of large genomic regions in *Vibrio cholerae*

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Detailed protocol

Natural transformation assay.

Natural transformation assays were performed by adding purified gDNA to the chitin-grown bacteria or by co-culturing the two non-clonal *V. cholerae* strains. To set up the experiments, the bacterial strains were grown as an overnight culture in LB medium at 30°C [growth in 3ml on rotating wheel for example].

After back dilution [1:20, so 50ul], the cells were incubated in the presence of chitin flakes (~80 mg; Sigma-Aldrich -[catalog number #9213]) submerged in a final volume of 1 ml of half-concentrated (0.5x) defined artificial seawater medium (Meibom *et al*., 2005).

When purified DNA served as the transforming material, 2 µg of the indicated gDNA (final concentration 2 µg/ml) was added after 24 h of growth on chitin (except for the experiments in which the gDNA was added at 0 h, as indicated in the text), and the cells were incubated for another 6 hours. [this all occured in Eppendorf tubes and under standing conditions].

At that point, the bacteria were detached from the chitin surfaces by vigorous vortexing [for 30 sec] and then were serially diluted [in 1x PBS buffer; but the same minmal medium that was used for the experiemnt works as well].

Colony-forming units (CFUs) were enumerated on selective (antibiotic-containing; [that is kanamycin 75ug/ml for strains carrying the *aph* casette or chloramphenicol 2.5ug/ml for those with *cat* casettes) or non-selective (plain LB) agar plates, and the transformation frequency was calculated by dividing the number of transformants by the total number of CFUs.

For mixed community assays, the two strains were inoculated simultaneously at a ratio of 1:1 in a final volume of 1 ml. We estimated that if all of the inoculated prey/donor cells would lyse, ~0.2 µg/ml of DNA would be released into the medium. These mixtures were incubated for 30 h before the bacteria were harvested, diluted, and plated, as described above (maximum prey/donor DNA that could be released at this point corresponds to ~0.4 µg/ml).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Blokesch, M. (2019). Natural transformation assay. Bio-protocol Preprint. bio-protocol.org/prep148.
2. Matthey, N., Stutzmann, S., Stoudmann, C., Guex, N., Iseli, C. and Blokesch, M.(2019). Neighbor predation linked to natural competence fosters the transfer of large genomic regions in *Vibrio cholerae*. eLIFE. DOI: 10.7554/eLife.48212